

STUDY ON ADSORPTION BEHAVIOR OF ALKALINE PROTEASE ON POLY(ETHER SULFONE) INTEGRATED WITH FISH SCALE HYDROXYAPATITE AS SELF ORGANIZED IN ION EXCHANGE MEMBRANE

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ABSTRACT

This study was performed to investigate the physiochemical effect on the protease adsorption produced by *Bacillus sp.* using highly specific ion exchange membrane (IExM). Effects of pH solution (adjusted with HCl and NaOH), ionic strength (adjusted by NaCl), and initial concentration on adsorption study were performed by using affinity polyethersulfone (PES) membrane integrated with fish scale hydroxyapatite (FSHAp), FSHAp/PES IExM. At 1.0 M, the maximum adsorption capacity was recorded by 0.229 mg/cm² in 90 minutes incubation time. The optimum pH was found to be at pH 8 with adsorption capacity of 0.211 mg/cm² in 90 minutes incubation time. The optimum adsorption capacity with 0.204 mg/cm² was observed when adsorption study performed with initial concentration at 0.9 mg/ml. Experimental data showed the protease adsorbed higher in alkaline condition compared to acidic solution. Further experiment showed that increase in ionic strength leads to noticeable increase in adsorption capacity which is due to aggregation of enzyme molecules in solution. The adsorption study also showed great adsorption behavior when high initial concentration applied as high number of free enzyme molecule to be adsorbed.

Key words: FSHAp, PES, IExM, pH, ionic strength, initial concentration, adsorption capacity

INTRODUCTION

As the effects of rapid developments in biotechnology and the pharmaceutical sector, the demand for highly purify polypeptides, proteins and also polynucleotides increased (Hancock *et al.*, 1992; Zou *et al.*, 2001). Many studies have reported the performance of adsorptive membranes, ion-exchange membranes, affinity membranes, reversed-phase membranes, and hydrophobic-interaction membranes, as well as their theoretical description and optimal design which used to purify the potential proteins (Zou *et al.*, 2001; Suen *et al.*, 1993). The integration of membrane and ion exchange chromatography provides a number of advantages over other chromatography especially in term of time and activity recovery (Suen *et al.*, 1993).

There are so many options for materials which are commercially available to choose from, and these include organic, inorganic, composite and polymeric materials as to prepare the basic membranes (Roper and Lightfoot, 1995). The polymeric material has been widely used as it can offer many advantageous compared to other options. Polyethersulfone (PES) is one of the most important polymeric materials which exhibit transparent and amorphous properties, high T_g (225°C), strong resistance to heat, chemicals as well as compaction by hydraulic pressure (Ahmad *et al.*, 2013). However, the major drawback of the membrane is related to its relatively hydrophobic character (Van *et al.*, 2008).

Advance researchers need to be conducted as to find and develop new membrane materials and structures specifically in perspective of reducing fouling effects, biocompatibility and function. Common methods used to achieve this are modification of the material itself, blending and

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surface modification (Van *et al.*, 2008; Zhao *et al.*, 2013). Basically, surface-coating is a method where a thin layer called selective layer which is directly layered as a coating of thin film layer on the top of membrane surface. This thin layer normally produced from material that have good hydrophilic characteristic (Zhao *et al.*, 2013).

Today, hydroxyapatite (HAp) is one of the new materials that are gaining so much attention to be used as adsorbent material, since it demonstrated good hydrophilic property (Luo and Joseph, 1998). HAp can be obtained from various sources such as fish bones (Panda *et al.*, 2013), bovine bones (Chakraborty, and Chowdhury, 2013) teeth and bones of pig (Zaragoza *et al.*, 2009). and fish scale (Xiaoying *et al.*, 2007; Kongsri, 2013) which has been considered as an environmental benign functional material. Due to its wide range applications, less sophisticated production with low cost HAp has attracted a great deal of attention today (Lari *et al.*, 2013). In recent years, further applications of HAp with multi-adsorbing sites have been found in the purification and separation of proteins, enzymes and other biological molecules since it has good adsorption to protein (Yang and Zhang, 2009; Sun and Jun, 2014). Protein separation is closely related to the interactions between proteins and various kinds of adsorbents (Sun and Jun, 2014; Nordstro *et al.*, 1999). Therefore, better understanding on this interaction behavior is very crucial.

In this study, PES was used for the preparation of the porous membrane matrix and HAp extracted from the fish scale omit as adsorptive particle. Protease produced by *Bacillus* sp. was used as a protein model in this work. Three physiochemical conditions; pH, ionic strength and initial protease concentration were studied.

MATERIALS AND METHODS

Materials

Poly (ether sulfone) (PES) (Mw= 58,000) was produced by BASF Company. The fish scales were collected from a local market in Kuala Terengganu province located at Northeastern Malaysia. The HAp was extracted from the fish scale by using the alkaline heat treatment method. Protease (Mw= 32 kDa) was supplied by China Medicine Chemical Reagent Company.

Fabrication of fish scale hydroxyapatite-polyether sulfone, FSHAp/PES IExM

Membrane dope was prepared by gradually adding 15 wt% of PES into heated NMP. The modification of the membrane was performed by using self assemble method. The native PES

membrane was immersed into the FSHAp solution with 0.8 %wt for 60 minutes in acetic acid at pH 5. The hybrid membrane was dried at room temperature overnight. Then, the dried membrane was neutralized with NaOH solution which has been prepared in 0.1 M in 50 % water-ethanol mixture for 30 minutes. The membrane was rinsed with 50%v ethanol solution for 3 times. The IExM was washed with distilled water. The IExM was stored in the distilled water for characterization and performance tests (Panda *et al.*, 2013; Kongsri, 2013).

Protease adsorption capacity

Protease adsorption was investigated within a range of pH from 5, 7, 8, 10 and 12 (phosphate buffer). The adsorption experiments were also carried out with different initial protein concentration as well as ionic strength by using mono-valent salt, NaCl. The membranes were dried at 30°C in a vacuum oven. The samples containing 100 mg/ml protease buffer solution were incubated for 30, 60, 90, 120 and 150 minutes with an exact amount of membranes disk cut into area for about 14.6 cm² in sealed containers under continuous shaking at 25°C. The FSHAp/PES IExM adsorbed the protease there by reducing the protease concentration in the bulk. After each incubation periods over, the equilibrium protease concentration were monitored with a UV-1800 spectrophotometer. The protease depletion was measured at 595 nm with 5 mm quartz cuvettes. The enzyme concentration was monitored by Bradford assay. The amount of adsorbed protease was obtained using the following equation (Bayramoglu *et al.*, 2013; Barka *et al.*, 2011).

$$q = \frac{(C_0 - C) V}{A} \quad (1)$$

Where q is the amount of adsorbed protease onto the membrane (mg/cm²); C_0 and C are the total protein in the initial solution and the aqueous phase after adsorption respectively (mg.ml⁻¹); V is the volume of the aqueous solution (ml); A is the area of the membrane used in the adsorption medium (cm²).

RESULTS AND DISCUSSION

Effects of pH values on protease adsorption

Fig 1 (a) shows the adsorption of protease on the pure HAp particles on PES/FSHAp at different pH value (5, 7, 8, 10 and 12). The protease adsorption capacities of pure HAp particles, increase with the increasing pH value in the solutions until it reach the maximum value of pH 8 (in 17 mM phosphate buffer) and started to decline gradually at pH 10 and 12. As compared to the protease

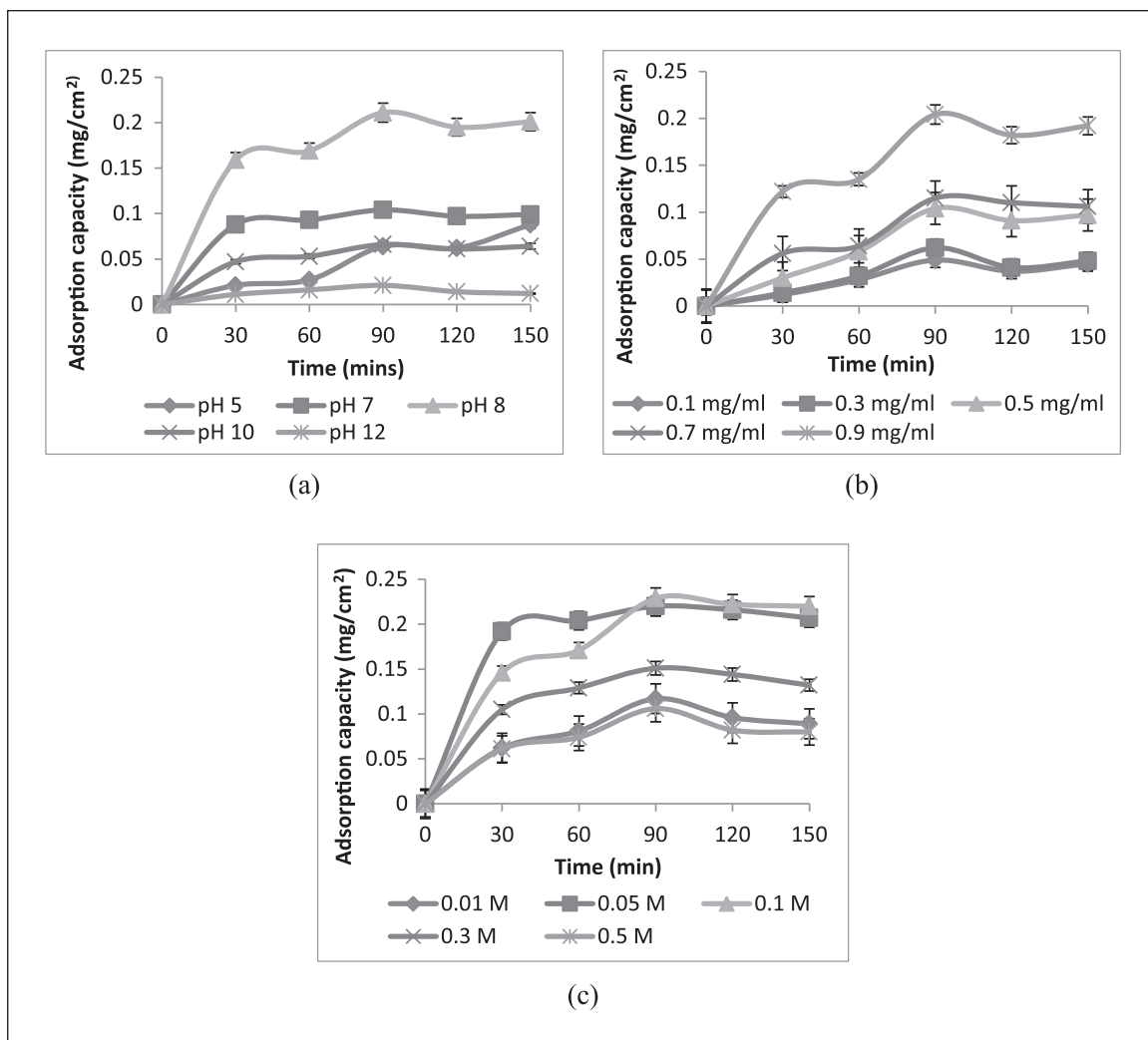


Fig 1: The adsorption kinetics of protease on PES/FSHAp IExM at different (a) pH value (b) initial concentration and (c) ionic strength in time.

adsorption capacity of FSHAp particles in the pH 8, the protease adsorption capacity of FSHAp showed the maximum value with 0.211 mg/cm² in 90 minutes incubation time. Declining in the adsorption capacity might be caused by the distortion of charge near the surface of FSHAp which reducing the protease adsorption efficiency (Kongsri, 2013).

The amount of total enzyme adsorbed is observed to be significantly influenced by the pH as it occurred mainly based on the electrostatic interaction (Okeola and Odebunmi, 2010; Yi *et al.*, 2003). Protease is adsorbed mainly through electrostatic attraction between the COOH group of protease and the calcium ion exposed on the surface of HAp (Klinger *et al.*, 1997). The adsorption process continues until the electrostatic repulsion between the free protease molecules in the medium and protease that have been adsorbed on the membrane surface reduce and limits the amount of the protease that can be adsorbed (Sun and Wu, 2014; Klinger

et al., 1997). The pH at the point of zero charge (pHPZC) of FSHAp has been previously reported to be 7.86 (Kongsri, 2013; Li *et al.*, 2007) and above this pH, HAp will possess negative surface charges, and its negative potential increases with the increase in pH (Li *et al.*, 2007).

At pH 8, attractive interactions are generated, resulting from the different charges between the negatively surface charges of FSHAp and positively charged amine groups of protease. FSHAp displayed slightly negative charged at pH 8 since this pH is above its IEP (Moradi and Zare, 2011). Meanwhile, the amine and amide groups of the protease ionized with H⁺, which leads the molecules of the protease to be positively charged (Barka *et al.*, 2011; Moradi and Zare, 2011). These protonated amino groups are readily to adsorb onto the negatively charged membrane surface as the effect of differential charges (Landi *et al.*, 2000). At pH 10, the poor performance might cause by the aggregation between these protease molecules since there is

almost no net charge or zero on the enzyme (Gustafsson *et al.*, 2013). Meanwhile at pH 12, the charge of enzyme molecule changed which in turn causing the enzyme to denature (Tripathi *et al.*, 2011). At pH 5, both of the pure HAp and enzyme possess the same charge, positive charge. This condition leads to the formation of a diffusive ion cloud that formed around the enzyme which resulting in decreasing of adsorption capacity (Aravind *et al.*, 2007).

Effects of ionic strength on protease adsorption

The adsorption mechanism also depends on the nature of solute-solute interaction as well as on salt concentration (Wan *et al.*, 2006). The effects of salt are different from each other due to the differences in charge density and smaller size of ion which will influence the way it move around in the solution thus prevent the adsorption process (Lee *et al.*, 2004). Adsorption capacity is hugely depends on the electrostatic interaction of adsorbate and adsorbent surface which in this study is referring to FSHAp and protease molecule or more specifically between Ca^{2+} cation and PO_4^{3-} anion of HAp particles with COO^- anion and NH_4^+ cation of protease protein, respectively (Wan *et al.*, 2006; Swain, 2013).

Fig 1 (b) shows the adsorption of protease on the pure HAp particles on PES/FSHAp at different ionic strength (0.01, 0.05, 0.1, 0.3 and 0.5 M). The protease adsorption capacities of pure FSHAp particles improved as the ionic strength value in the solutions increasing until it reach the maximum at 0.1 M (in 17 mM phosphate buffer) and started to decrease gradually at 0.3 and 0.5 M. In this study, the optimum adsorption was recorded to be at salt concentration of 0.1 M. The adsorption capacity that managed to be absorbed by the membrane is 0.229 mg/cm^2 in 90 minutes incubation time. Generally, the effect of NaCl concentration on the dynamic adsorption capacity is not significant when the NaCl concentration is low due to lower electrostatic exclusion interaction between the enzyme and membrane (Ferdous *et al.*, 2012).

The increase in NaCl concentration will reduce negative potential of FSHAp on the membrane surface which consequently causing the electrostatic repulsion force between FSHAp and protease to be reduced (Moradi and Zare, 2011; Spongberg and Lou, 2000). This explains why at 90 minutes of adsorption time, the optimum adsorption capacity recorded by the membrane is only 0.151 and 0.106 mg/cm^2 at ionic strength of 0.3 and 0.5 M respectively. Further increased ionic strength will induce salting out effect caused the protease to agglomerate and its solubility in salt solution to be decreased (Spongberg and Lou, 2000).

Effects of initial protease concentration on protease adsorption

In this study, the effects of initial protease concentration were carried out as it is one of the most important physiochemical to study. This is because the effect of an initial concentration can overcome all mass transfer restrictions of protease molecule between the aqueous and solid phases (Bayramoglu *et al.*, 2006). The adsorption capacity of protease molecule at different initial concentrations was illustrated in Figure 1. This Fig. 1(c) shows plots of equilibrium adsorption capacity versus the various initial concentration of protease molecule on IExM.

It is clear that the equilibrium adsorption capacity increased with the increasing of initial concentration at a constant time because of the high driving force, and reaches saturation when the equilibrium concentration of protease increases to at about 1.5 mg/ml because the adsorption sites of FSHAp have been occupied totally. This indicates that higher initial concentration of protease can enhance the adsorption process (Hou *et al.*, 2012; Kongsri *et al.*, 2013). In this study it found that 90 minutes is the optimum time for adsorption to take place and after 90 minutes, almost all the protease molecule was bound to FSHAp deposited on the membrane.

CONCLUSIONS

This study reveals the potential of using PES/FSHAp IExM as an excellent low-cost method for the protease purification from aqueous solutions. At 1.0 M and pH 8, the maximum adsorption capacity was recorded by 0.229 and 0.211 mg/cm^2 respectively. With initial concentration at 0.9 mg/ml , the optimum adsorption capacity recorded with 0.204 mg/cm^2 . Equilibrium adsorption data are crucial for evaluating the properties of adsorbents since it can provide important factors pertain to large-scale chromatographic operations for protein purification.

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